

US EPA/ORD PFAS Analytical Methods

- *Mark Strynar – US EPA National Exposure Research Laboratory*



December 14th, 2016
Colorado School of Mines
On-Site Analytical Workshop

NERL/EMMD developed methods for PFAS analysis (2005-2016)

(Strynar/Lindstrom)

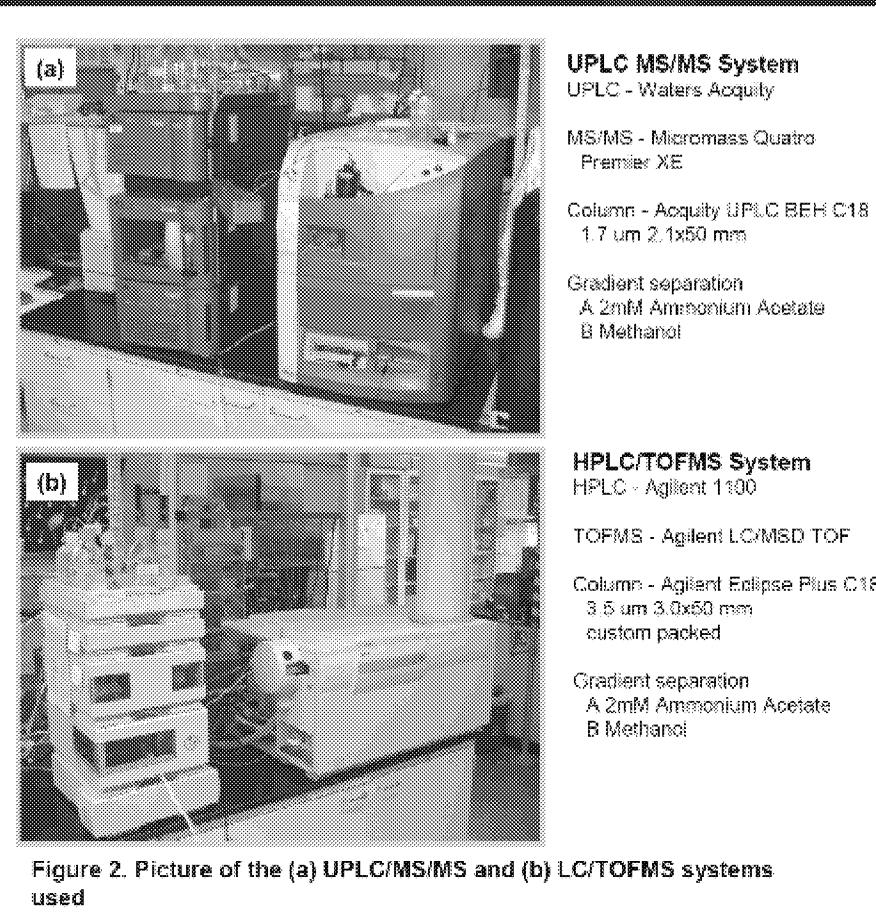
Peer reviewed publications on all media

- surface/ground/waste-water
- soil/sediment
- whole-fish homogenate, fillet, livers
- serum (human, bald-eagle, feline, and dosed rodent)
- dosed rodent (urine, mammary gland, liver, whole pup)
- house dust

Typical Analysis of Samples for PFAS

- Matrix matched (when possible) extracted standard curves with stable isotope dilution quantitation
- As many matched stable isotope labeled internal standards as possible to account for matrix interference and analyte recovery
- High level of QA/QC (~10% duplicates, method blanks, solvent blanks QA/QC pools or NIST SRM samples (when available)
- Typical sensitivity LC-MS/MS methods is 1-10 pg OC.
- LOQ is method specific

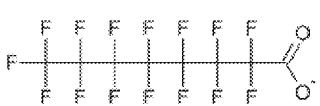
Analytical Equipment



- Low resolution
 - Nominal mass
 - Chevy
-
- High resolution
 - Exact mass
 - Ferrari

Figure 2. Picture of the (a) UPLC/MS/MS and (b) LC/TOFMS systems used

Some Typical PFAS



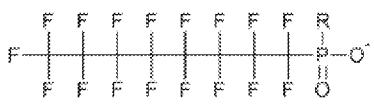
Perfluorocarboxylic acids (ex. PFOA)



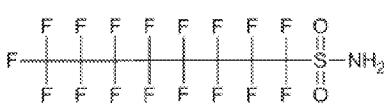
Perfluorosulfonic acids (ex. PFOS)



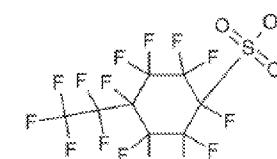
Fluorotelomer alcohol
(ex. 8:2 FTOH)



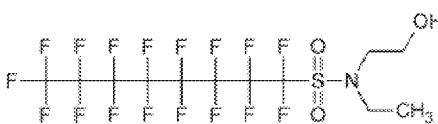
Perfluorophosphonic/phosphinic acids
(ex. If R=OH then PFOPA
If R=C8 perfluoroalkane then 8:8 PFPI)



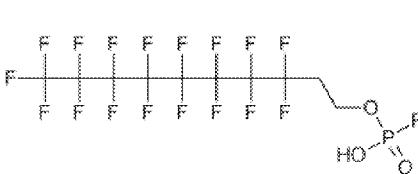
Perfluorosulfonamide
(ex. FOSA)



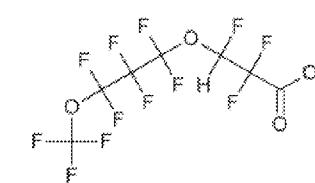
Perfluorinated cyclo sulfonates
(ex. PFECHS)



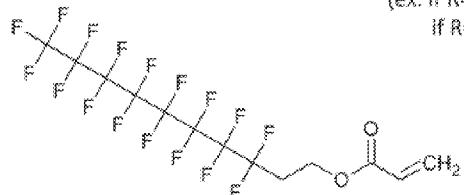
Perfluorosulfonamidoethanol
(ex. N-EtFOSE)



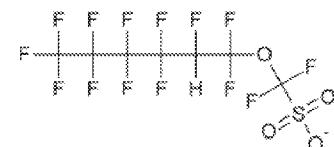
Fluorotelomer phosphate esters
(ex. if R= OH then 8:2 monoPAP
If R= 8:2 FTO ester then 8:2 diPAP)



Polyfluorinated ether carboxylates
(ex. 4,8-dioxa-3H-perfluorononanoate)



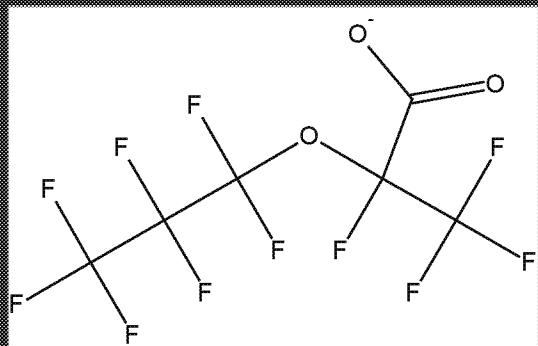
Polyfluorinated polymeric unit
(ex. 1H,1H,2H,2H-perfluorodecyl acrylate)



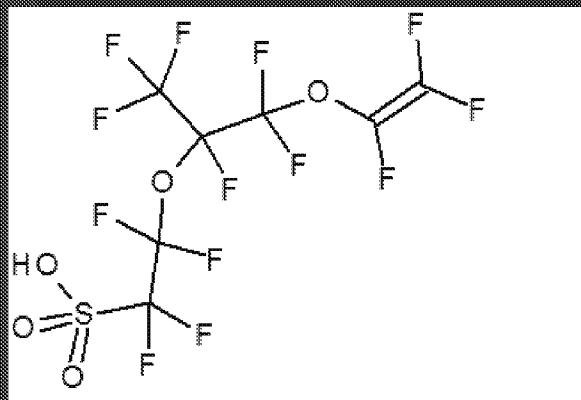
Polyfluorinated ether sulfonates
(ex. Perfluoro [hexyl ethyl ether sulfonate])

Some newly discovered PFAS NERL/EMMD

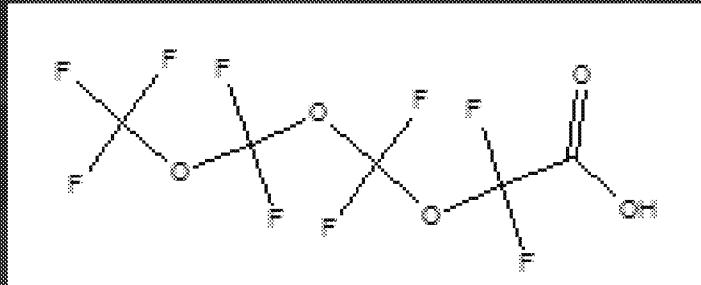
Cape Fear River NC; Strynar et al., 2015 ES&T



GenX plus 5 related



Nafion +1 related



Polyether fluoros plus 3 related

Sun et al., 2016 ES&T Letters
Concentration and GAC removal efficiency
in DW

Contamination Issues

PFAS Analytical Lessons Learned

- “When in doubt assume PFAS contamination is the culprit”

Equipment/Materials we found to have PFAS

PTFE lined septa

Most common contaminants

PTFE lines

C6, C7, C8

Solvents

(carboxylates)

Low binding pipette tips (C6-C12)

Polypropylene vials, caps, centrifuge tubes

Blank matrix (serum, fish, livers.....)

Preparatory/Analytical Equipment

Recommended vials/tubes/tips

BD Falcon Tubes (sterile or nonsterile) 15 ml or 50 ml

Gennesse pipette tips

Triple rinse with methanol (last resort)

Problems with analysis of PFAS at trace levels

PFAS are found in all sorts of lab equipment and supplies

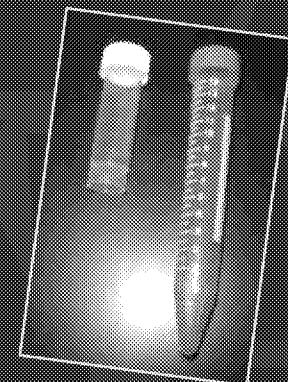
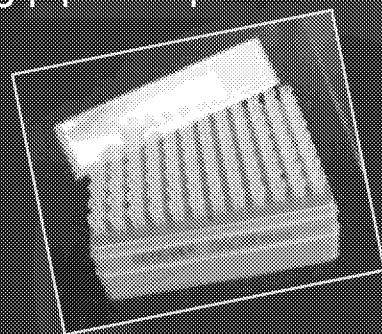
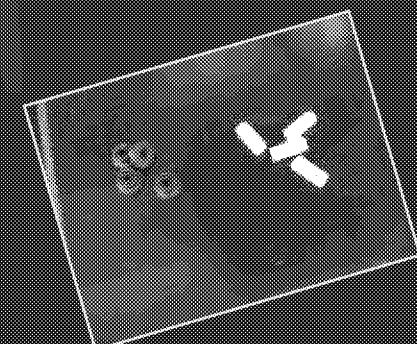
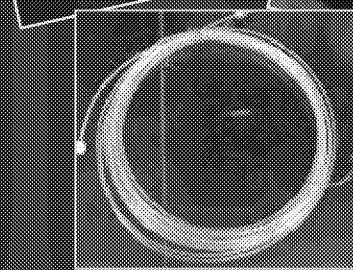
Solvents (methanol, acetonitrile)

PP vials and centrifuge tubes (mold release)

LC vial caps septa (PTFE/silicone)

LC and SPE pump equipment
pump head seals (graphitized Teflon)
PTFE tubing

Low binding pipette tips

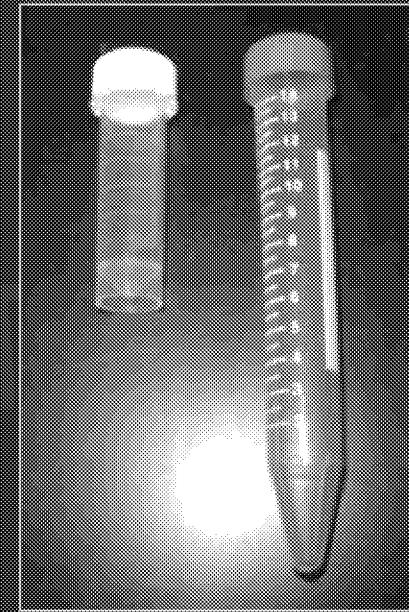
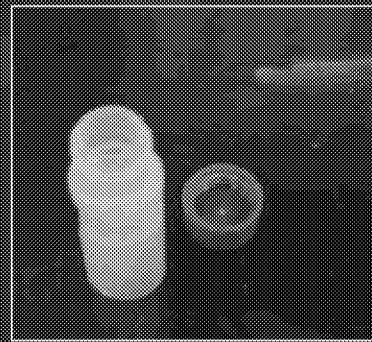


Problems with analysis of PFAS at trace level contd.

A Blank matrix (water, serum, soil, house dust, fish) is difficult to find
-necessary for QC pool or matrix matched standard curves

Blanks are used to gauge day to day contamination issues
double blanks (solvent blank, no matrix no I.S.)
method blank (no matrix, I.S.)
matrix blank (matrix, I.S.)

Biggest issue is intermittent contamination



Instrumentation Issues

Analytical equipment **MAY** need to be altered



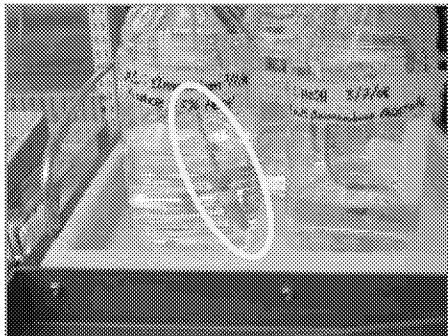
LC/MSD TOF
HPLC Agilent 1100
MSD TOF Agilent 1969A
Capabilities accurate 1-10 ppm
-pg OC



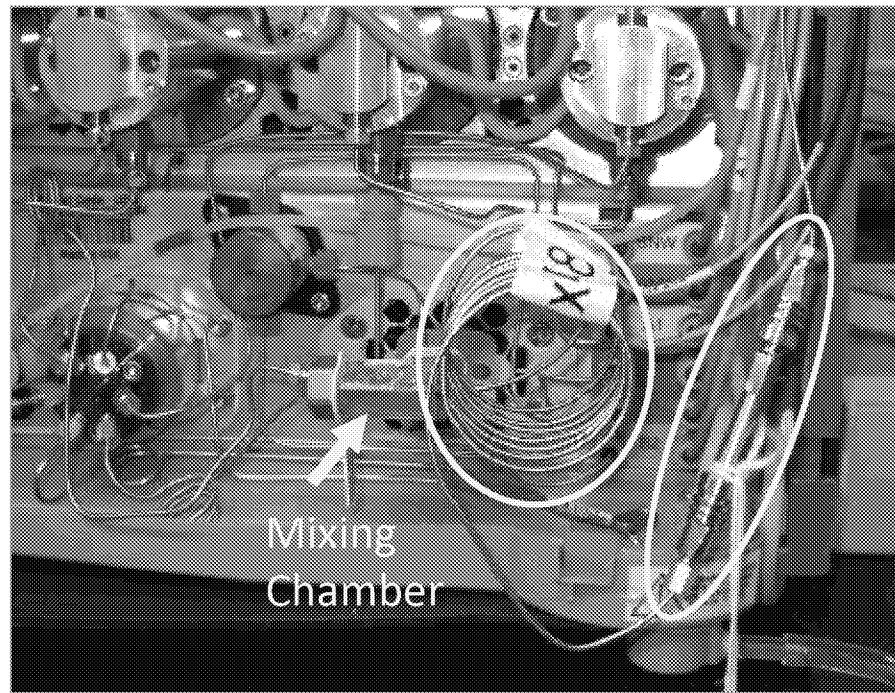
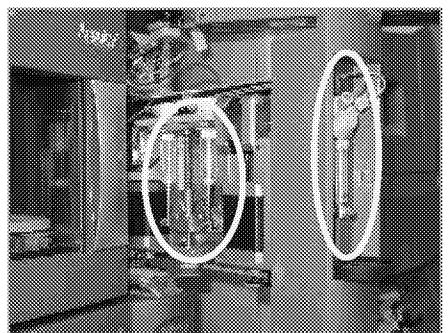
LC-MS/MS
UPLC Waters Acquity Modifications
MS/MS Quattro Premier XE
Capabilities low pg – high fg OC

System Modifications: Instrumental background noise – gradients separations

PEEK/SS filters

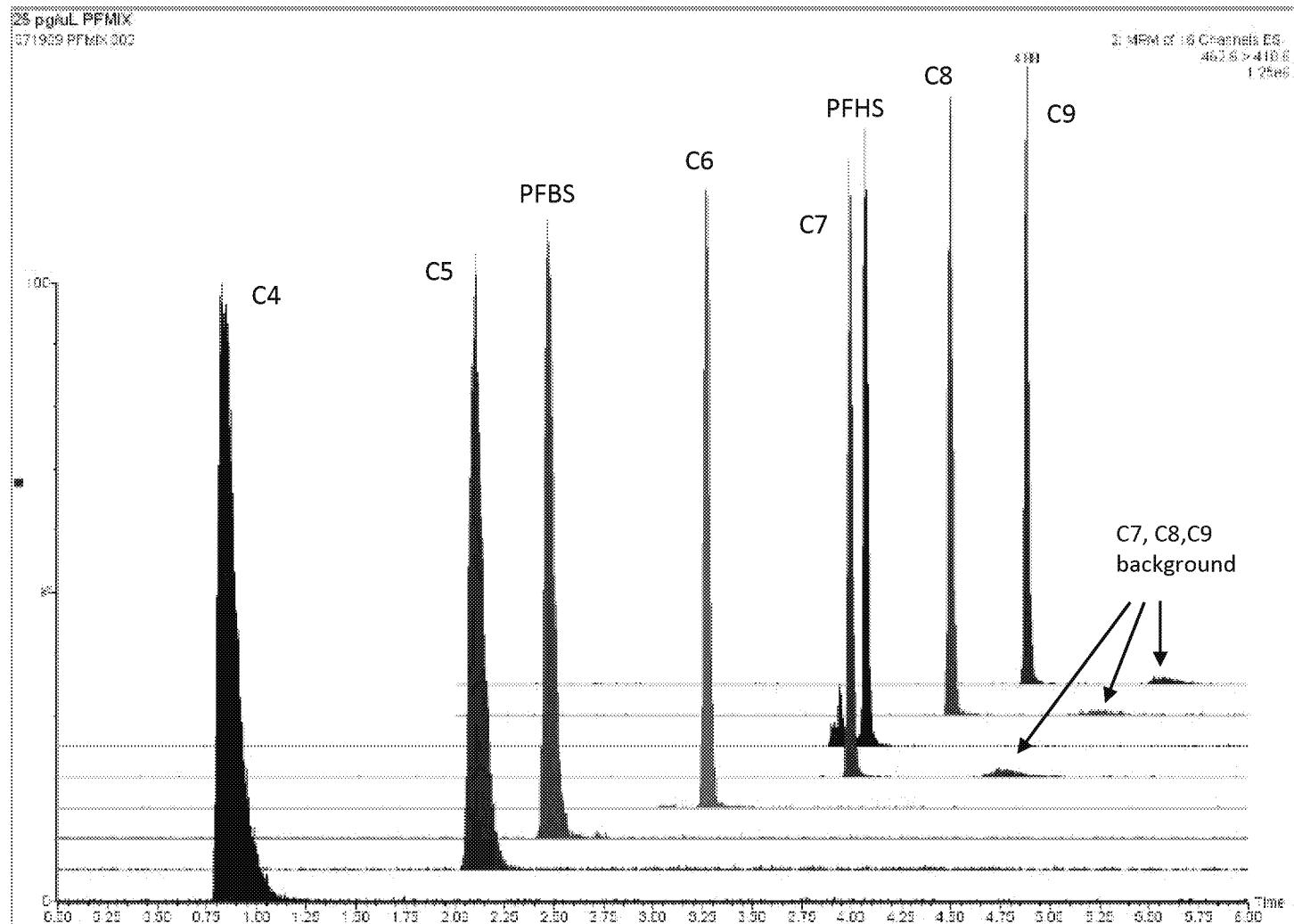


No changes can be made



Critical in separating instrument contamination for analytical peaks

Hold up column and standards



Study Plan for Multi-laboratory Validation of Two EPA Methods for the Analysis of Perfluorinated Alkyl Substances (PFAS) Part I: Waters

Lead: EPA/OLEM (Terry Smith)

1) Two water methods (wastewater, surface water, ground water, reagent water)

Chicago Regional Laboratory EPA Region 5 ASTM International methods (ASTM D7968-14, Ref. 8.5 and ASTM D7979-15, Ref. 8.6) for the analysis of select PFAAs in soil and water matrices (Lawrence Zintek-R5-Direct Injection)

ORD/NERL (Strynar/Lindstrom) water samples (EMAB-114.0) and (MDAB 066.0 surface soils) (Strynar – ORD isotope dilution)

2) Two soil/sediment methods



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Analytes (n=24 native; n=19 isotope labeled)

Wellington PFAC-24PAR (2 ng/uL) \$250/1.2 mL

	Analyte Name	Acronym	CAS Number
N-1	Perfluorotetradecanoic acid	PFTreA	376-06-7
N-2	Perfluorotridecanoic acid	PFTriA	72629-94-8
N-3	Perfluorododecanoic acid	PFDoA	307-55-1
N-4	Perfluoroundecanoic acid	PFUnA	2058-94-8
N-5	Perfluorodecanoic acid	PFDA	335-76-2
N-6	Perfluorononanoic acid	PFNA	375-95-1
N-7	Perfluoroctanoic acid	PFOA	335-67-1
N-8	Perfluoroheptanoic acid	PFHpA	375-85-9
N-9	Perfluorohexanoic acid	PFHxA	307-24-4
N-10	Perfluoropentanoic acid	PPeA	2706-90-3
N-11	Perfluorobutanoic acid	PFBA	375-22-4
N-12	Perfluorodecanesulfonate	PFDS	335-77-3
N-13	Perfluorononanesulfonate	PFNS	68259-12-1
N-14	Perfluoroctanesulfonate	PFOS	1763-23-1
N-15	Perfluoroheptanesulfonate	PFHpS	375-92-8
N-16	Perfluorohexanesulfonate	PFHxS	355-46-4
N-17	Perfluoropentansulfonate	PPeS	2706-91-4
N-18	Perfluorobutanesulfonate	PFBS	375-73-5
N-19	Perfluoroctanesulfonamide	PFOSA	754-91-6
N-20	Fluorotelomer sulfonate 8:2	FtS 8:2	39108-34-4
N-21	Fluorotelomer sulfonate 6:2	FtS 6:2	27619-97-2
N-22	Fluorotelomer sulfonate 4:2	FtS 4:2	NA
N-23	N-ethyl-N-((heptadecafluoroctyl)sulfonyl)glycine	NEtFOSAA	2991-50-6
N-24	N-(Heptadecafluoroctylsulfonyl)-N-methylglycine	NMeFOSAA	2355-31-9



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Analytes (n=24 native; n=19 isotope labeled)

Wellington MPFAC-24ES (1 ng/uL) \$700/1.2 mL

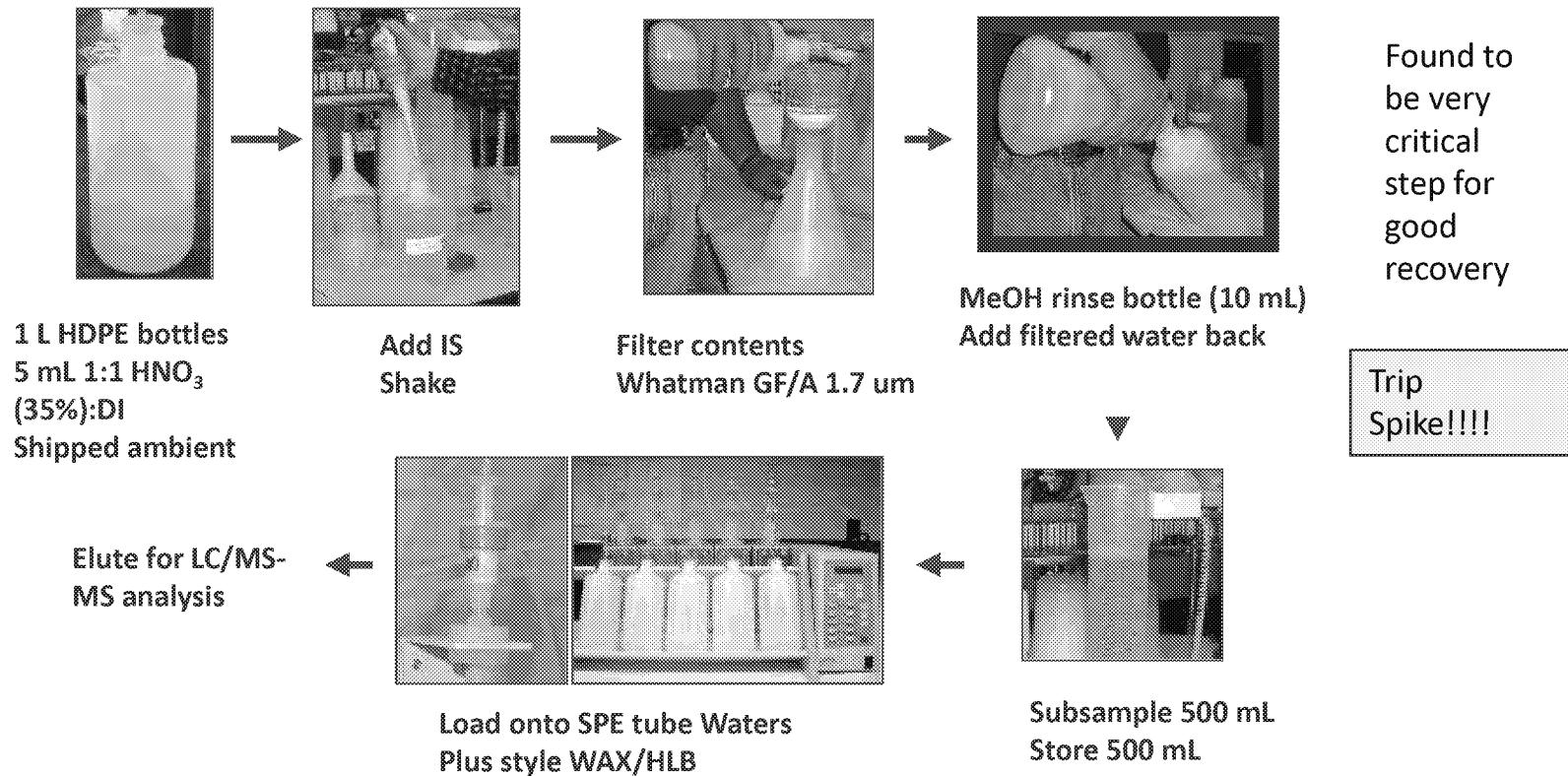
	Analyte Name	Acronym
	PERFLUOROALKYLCARBOXYLATES (PFCAs)	
L-1	Perfluoro-n-[1,2- ¹³ C ₂]tetradecanoic acid	M2PFTeDA
L-2	Perfluoro-n-[1,2- ¹³ C ₂]dodecanoic acid	MPFD ₀ A
L-3	Perfluoro-n-[1,2,3,4,5,6,7- ¹³ C ₇]undecanoic acid	M7PFUdA
L-4	Perfluoro-n-[1,2,3,4,5,6- ¹³ C ₆]decanoic acid	M6PFDA
L-5	Perfluoro-n-[¹³ C ₉]nonanoic acid	M9PFNA
L-6	Perfluoro-n-[¹³ C ₈]octanoic acid	M8PFOA
L-7	Perfluoro-n-[1,2,3,4- ¹³ C ₄]heptanoic acid	M4PFHpA
L-8	Perfluoro-n-[1,2,3,4,6- ¹³ C ₅]hexanoic acid	M5PFHxA
L-9	Perfluoro-n-[1,2,3,4,5- ¹³ C ₅]pentanoic acid	M5PFPeA
L-10	Perfluoro-n-[1,2,3,4- ¹³ C ₄]butanoic acid	MPFBA
	PERFLUOROALKYLSULFONATES (PFASs)	
L-11	Sodium perfluoro-[¹³ C ₈]octanesulfonate	M8PFOS
L-12	Sodium perfluoro-1-[1,2,3- ¹³ C ₃]hexanesulfonate	M3PFHxS
L-13	Sodium perfluoro-1-[2,3,4- ¹³ C ₃]butanesulfonate	M3PFBS
	PERFLUOROOCTANESULFONAMIDES (FOSAs)	
L-14	Perfluoro-1-[¹³ C ₈]octanesulfonamide	M8FOSA-I
	TELOMER SULFONATES	
L-15	Sodium 1H,1H,2H,2H-perfluoro-1-[1,2- ¹³ C ₂]decane sulfonate (8:2)	M2-8:2FTS
L-16	Sodium 1H,1H,2H,2H-perfluoro-1-[1,2- ¹³ C ₂]octane sulfonate (6:2)	M2-6:2FTS
L-17	Sodium 1H,1H,2H,2H-perfluoro-1-[1,2- ¹³ C ₂]hexane sulfonate (4:2)	M2-4:2FTS
	PERFLUOROOCTANESULFONAMIDOACETIC ACIDS	
L-18	N-ethyl-d5-perfluoro-1-octanesulfonamidoacetic acid	d5-N-EtFOSAA
L-19	N-methyl-d3-perfluoro-1-octanesulfonamidoacetic acid	d3-N-MeFOSAA

NERL/EMMD Water Analysis Method

Surface Water
Ground Water
WWTP Effluent
Drinking Water



Method Summary – Sample Processing



Nakayama et al., 2007 Environ. Sci.
Technol. 2007, 41, 5271-5276

Samples That Exceed Calibration Curve

Estimate concentration based on exceedance analysis

Dilute sample (analyte and IS presence) with DI lab water (IS added)
(ex 1:1, 2:1, 5:1 10:1) aiming for midpoint of calibration curve.

Example: Standard curve 10 – 1000 ng/L PFOA
Sample estimated to be 1600 ng/L

Dilute sample into new HDPE bottle:
1 part (250 mL prepared water sample with IS spike and analytes)
1 part(250 mL) DI water with IS spike at same level as real sample
Re-extract, elute and analyze.

Calibrate against existing calibration curve.

Source of Analytical Standards

Stable Isotope Labeled (^{13}C , ^{18}O , D):

Wellington Labs

Cambridge Isotopes Labs



Native:

Wellington Labs (Mixtures – 1-2 ng/uL; Individual 50 ng/uL)

Sigma-Aldrich

Synquest Labs

Oakwood Products

Manufacturer (industrial mixtures 3M, DuPont,
Mason Chemicals)



Method Summary – Water Sample Collection

SS Kemmerer sampler



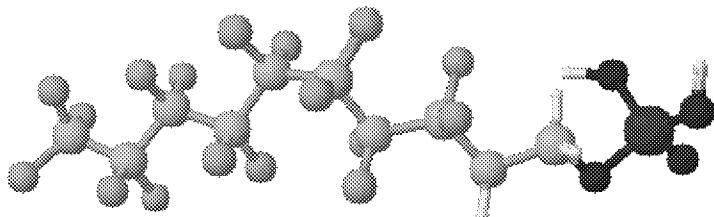
Lab-made dip sampler



Nalgene 1L HDPE bottles: PFAS free; Prefer wide mouth bottles

Quality Assurance – Quality Control

Trip spike (50 ng/L) MOST PEOPLE DO NOT DO THIS
Trip blank (DI)
Lab QC spikes (5 and 50 ng/L) – secondary source
Replication (>10%)
Occasionally standard addition



Target Compounds

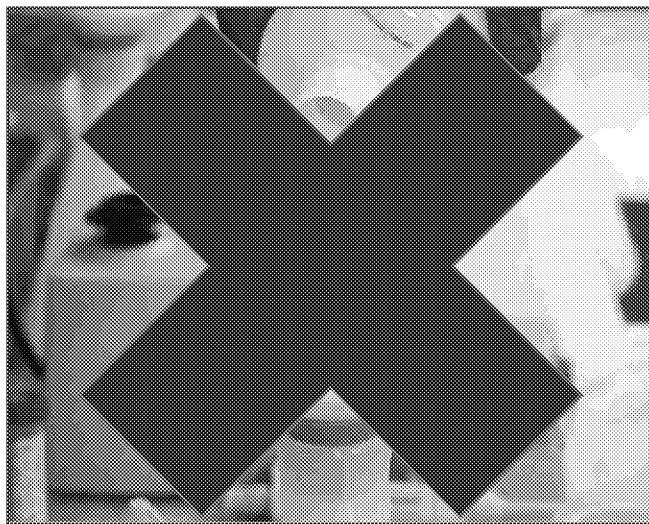
C₄-C₁₀ carboxylic acids

PFBS, PFHS, PFOS, PFDS

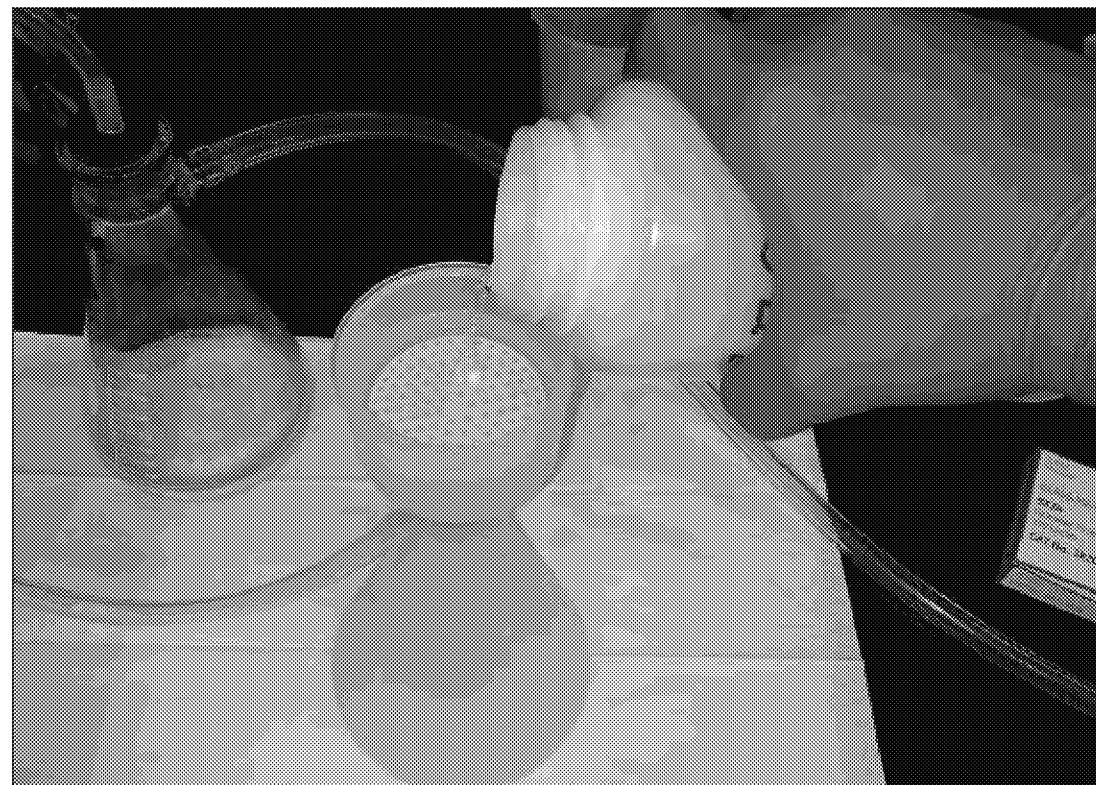
Internal Standards

¹³C₄-PFBA, ¹³C₂-PFHxA, ¹⁸O₂-PFHxS,

¹³C₂-PFOA, ¹³C₄-PFNA, ¹⁸O₂-PFOS, ¹⁸O₂-PFHxS,



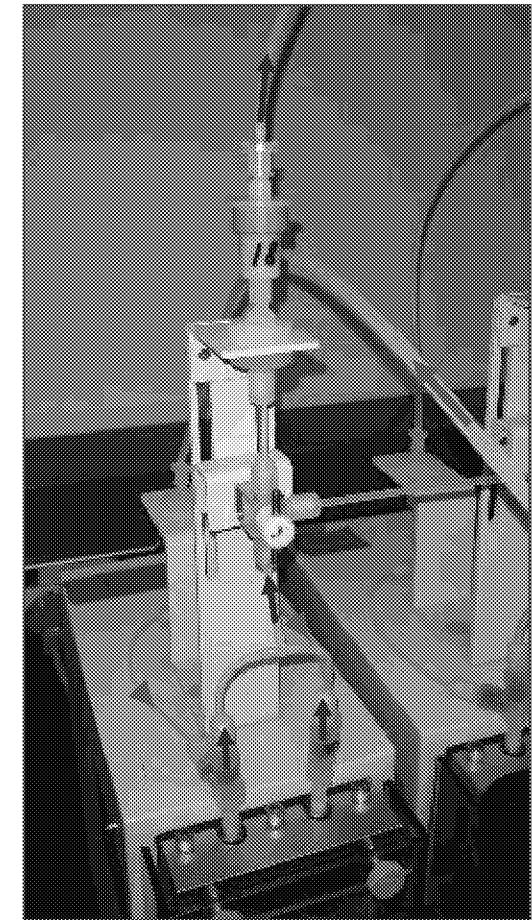
Sample Filtration



Positive Displacement Sample Concentration



CHARTEC Sep-Pak Concentrator
SPC 10-C (10 ml/min)



Waters Oasis WAX-plus

Sample Elution



Note: Rack modified to fit 15 mL BD Falcon tubes

Wash:

4 mL pH 4.0 buffer
(Acetate/Acetic Acid water)

4 mL methanol

Elute:

4 mL NH₄OH/MeOH

Evaporate:

~15-20 minutes 40°C N₂
TurboVap concentrator

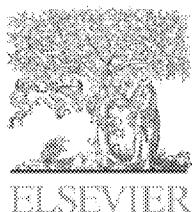
~ 0.5 – 1.0 mL

Sample prep for analysis:
Prepare sample to match
starting conditions of UPLC
gradient (75:25 aqu:MeOH)

NERL/EMMD Soil Analysis Method

Surface Soil
Subsurface Soil
House-dust
Sediment

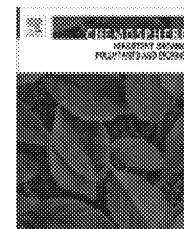
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Pilot scale application of a method for the analysis of perfluorinated compounds in surface soils

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Perfluorinated Compounds in surface soils

MDAB 066.0; Strynar et al., 2009



Office of Research and Development

National Exposure Research Lab/ Human Exposure and Atmospheric Science Division

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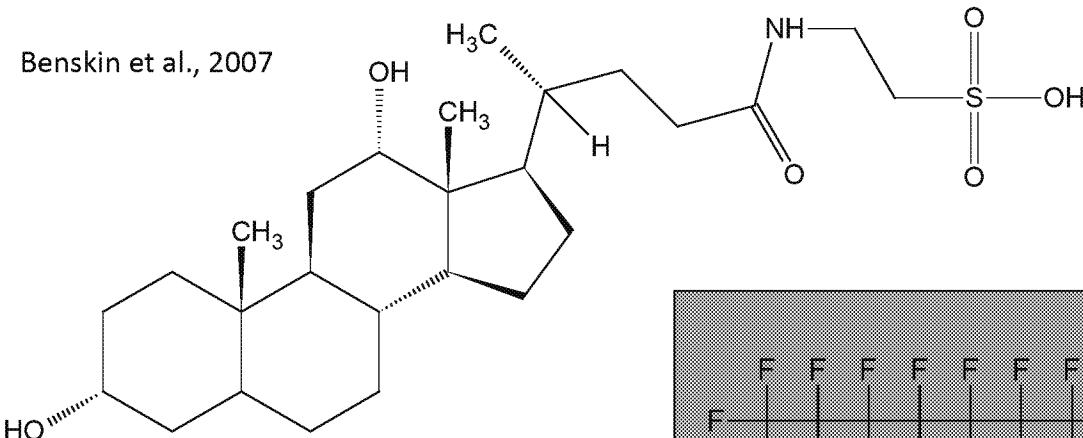
Analytes and Isotope Labeled PFAS

Table S1. Perfluorinated analytes, internal standards, monitored transitions and ion ratios

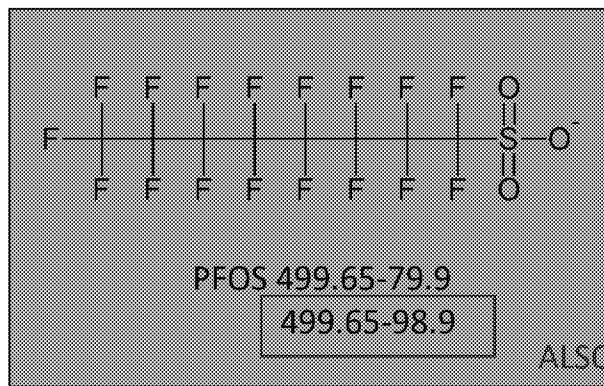
Analyte	Quantitation ion	Confirmation ion	Assigned IS	ion ratio* (mean)	ion ratio (SD)
PFHxA	312.70 > 268.70	312.70 > 118.70		17.67	2.72
PFHpA	362.65 > 318.70	362.65 > 168.65	¹³ C ₂ -PFHxA	5.33	0.41
PFOA	412.60 > 368.65	412.60 > 168.70		4.57	0.58
PFNA	462.60 > 418.60	462.60 > 218.75	¹³ C ₂ -PFOA	4.05	0.34
PFDA	512.60 > 468.55	512.60 > 468.55		6.81	1.10
PFUdA	562.60 > 518.60	562.60 > 268.70		6.32	0.92
PFDoA	612.60 > 568.55	612.60 > 168.75	¹³ C ₂ -PFUdA	10.95	1.49
PFTeDA	662.65 > 618.60	662.65 > 168.75		9.21	1.73
PFTrDA	712.65 > 668.65	712.65 > 318.80		12.29	2.61
PFBS	298.70 > 79.90	298.70 > 98.80		1.58	0.14
PFHS	398.65 > 79.90	398.65 > 98.80	¹⁸ O ₂ -PFHS	0.86	0.08
PFOS	498.65 > 79.90	498.65 > 98.80	¹⁸ O ₂ -PFOS	0.63	0.05
PFDS	598.60 > 79.90	598.60 > 98.90		0.42	0.06
¹³ C ₂ -PFHxA	314.75 > 269.75	NA			
¹⁸ O ₂ -PFHS	402.65 > 83.90	NA			
¹³ C ₂ -PFOA	414.75 > 369.70	NA			
¹⁸ O ₂ -PFOS	502.60 > 83.90	NA			
¹³ C ₂ -PFUdA	564.60 > 519.65	NA			

Known Interferences:

Taurodeoxycholic acid (TDCA) ENVICARB REMOVES ISSUE



MW 499.35-79.8
499.35-106.8



Preferred MRM
ALSO removes issue

Found in fish liver, fillets, avian eggs, human serum (SRM 1957), calf serum, cat serum, Bald Eagle blood ...Strynar et al., 2009 ASMS

Summary Soil Extraction Method

Sieve moist soil 2mm

Extract ~2 g moist soil
With 10 ml MeOH with IS mix

Subsample 2 g for moisture
content analysis (24 hr, 100 C)

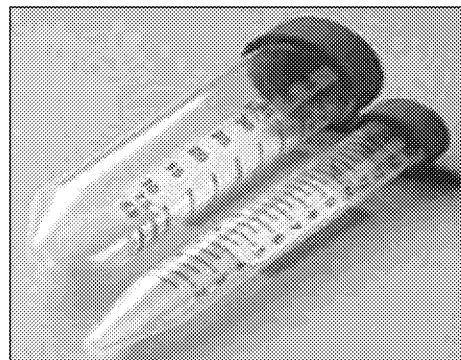
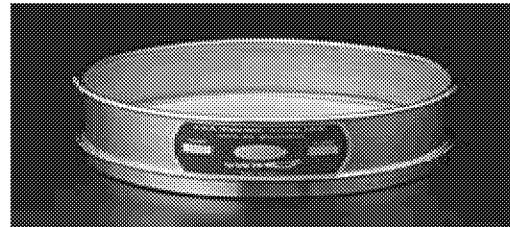
30 minutes shaking/sonication

10 minutes centrifuge (10k rpm)

Pass MeOH extract
Through graphitized carbon
SPE cartridge Supelco ENVI-
Carb (3cc-0.25 g)

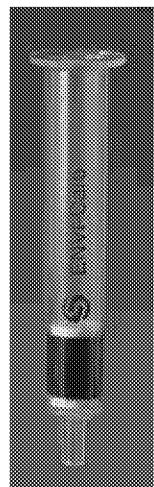
Evaporate to 0.5 mL

UPLC-MS/MS



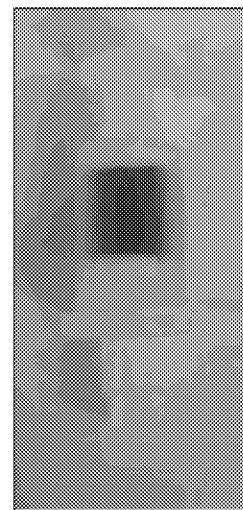
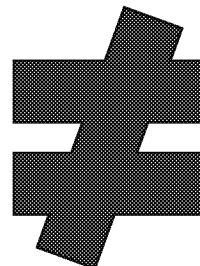
ENVI-Carb vs ENVI-Carb Plus

Supelclean ENVI-Carb



3cc or 6 cc

Supelclean ENVI-Carb Plus

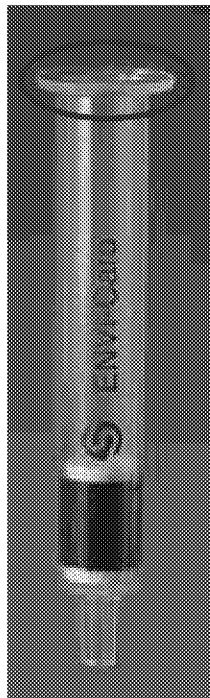


"Unlike traditional graphitized carbon black (e.g., Supelclean ENVI-Carb) which are granular and friable, Supelclean ENVI-Carb Plus consists of strong spherical carbon particles (carbon molecular sieve)...."

https://www.sigmaaldrich.com/content/dam/sigma-aldrich/docs/Supelco/Product_Information_Sheet/t708003.pdf

ENVI-Carb 3cc reservoirs

Supelclean ENVI-Carb
(3cc only)

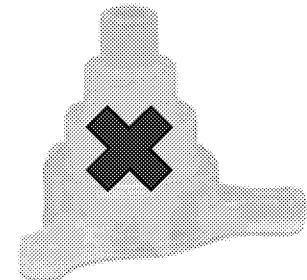


ID



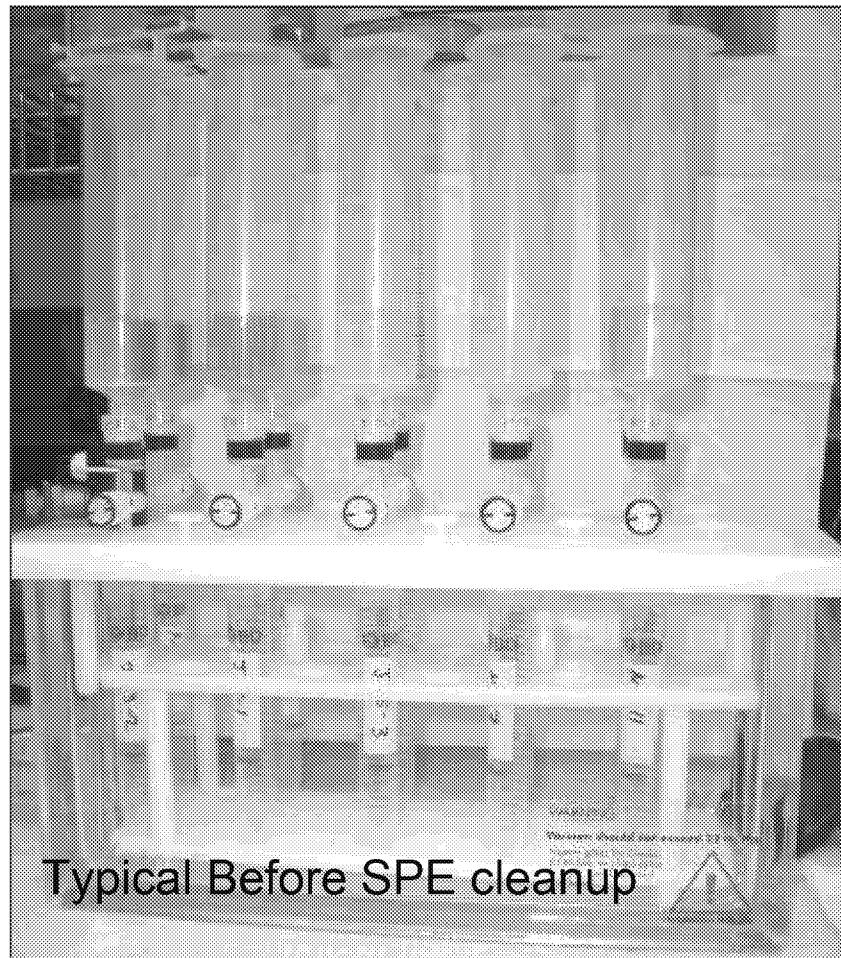
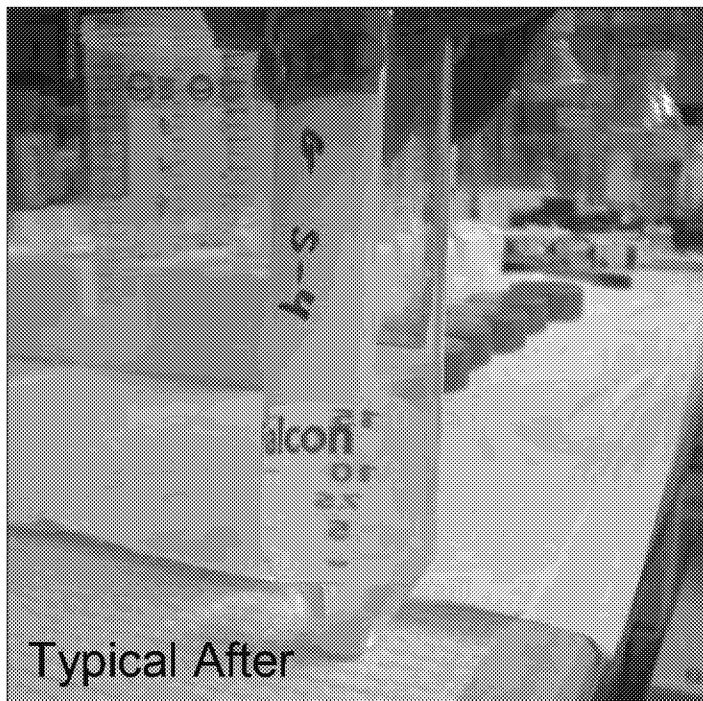
OD

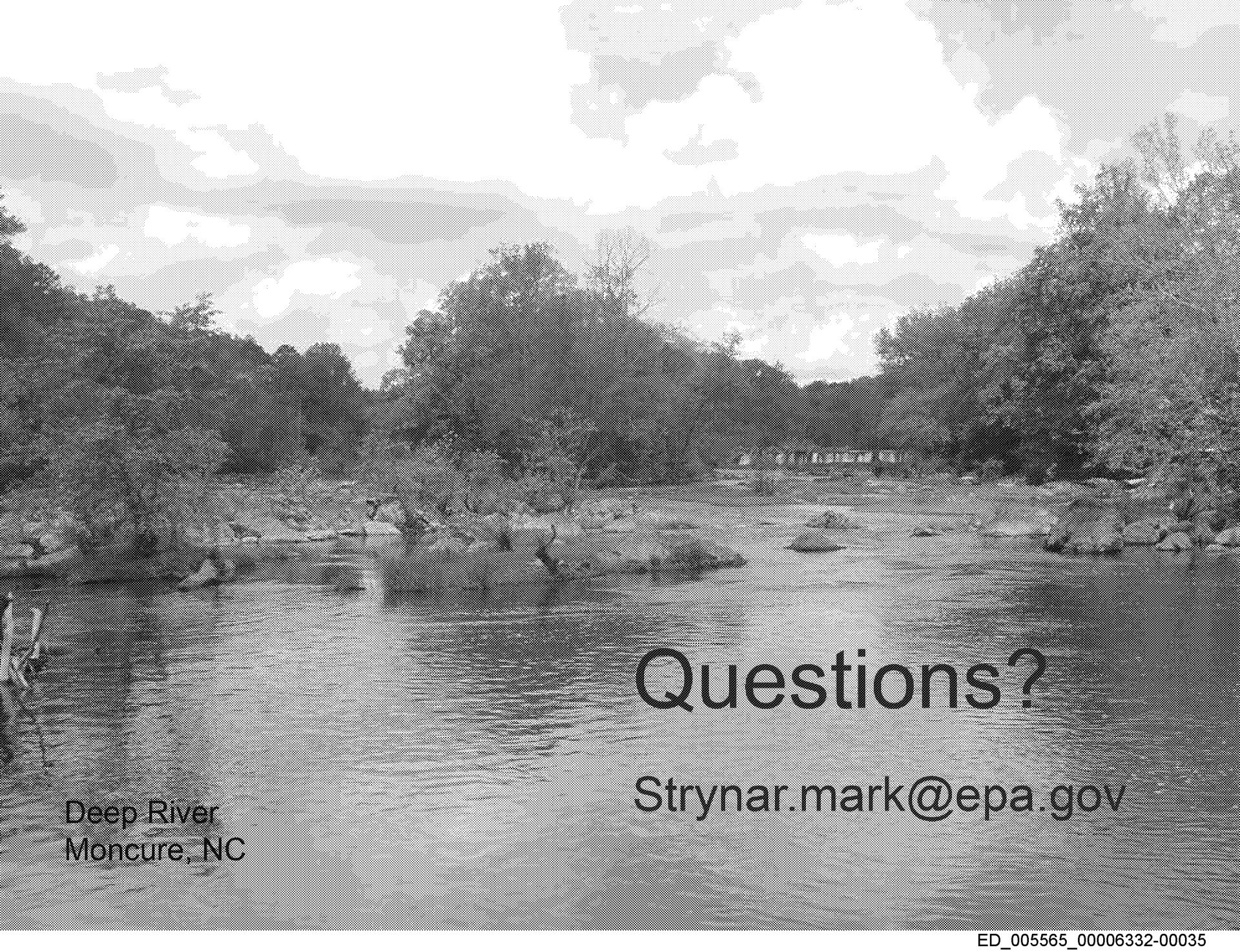
BD General Purpose
Luer-Lock Syringe
5 mL
10 mL
20 mL
60 mL



Soils from: (n=~300)

United States
China
Japan
Norway
Greece
Mexico





Deep River
Moncure, NC

Questions?

Strynar.mark@epa.gov